

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Tapas Mukhopadhyay, *et al.*

Serial No.: 10/043,877

Filed: January 9, 2002

For: ANTIHELMINTHIC DRUGS AS A
TREATMENT FOR
HYPERPROLIFERATIVE DISEASES

Group Art Unit: 1642

Examiner: B. J. Fetterolf

Atty. Dkt. No.: INRP:095US

**SECOND DECLARATION OF TAPAS MUKHOPADHYAY, SUNIL CHADA, ABNER
MHASHILKAR, AND JACK A. ROTH UNDER 37 C.F.R. §1.131**

We, Tapas Mukhopadhyay, Sunil Chada, Abner Mhashikar, and Jack A. Roth, hereby declare as follows:

1. We are the joint inventors of the subject matter claimed in the above-referenced patent application, U.S.S.N. 10/043,887, filed January 9, 2002.
2. We are submitting this declaration to set forth facts demonstrating that we both conceived the idea of the invention as reflected in the claims of the above-referenced patent application and determined that it functioned, prior to March 9, 1999.
3. Submitted as Exhibit 1 to this declaration is a copy of a FACS assay showing our experiments and results, entitled figures "1A" and "1B" which was prepared prior to March 9, 1999.

4. Submitted as Exhibit 2 to this declaration is a copy of our experiments and results in a study of the treatment of p53 wild type lung cancer cells with fenbendazole, which took place prior to March 9, 1999.

5. Exhibit 1 shows the results of our cell cycle analysis involving A549 (p53 wild type) non-small cell lung cancer (NSCLC) cells that have been treated with fenbendazole. The results show that the untreated A549 cells (A549C), have a standard profile of cells in various phases of the cell cycle, G1/S/G2, indicating a dominant G1 population. In contrast, the fenbendazole treated cells (A549 7EN) show a depression of both G2 and S phases and a G1 block. Furthermore, the fenbendazole treated cells show a distinct sub-G0-G1 population indicative of apoptotic cells. We generated the results of this cell cycle analysis prior to March 9, 1999.

6. Exhibit 2 shows the results of our study of the treatment of p53 wild type lung cancer with fenbendazole. We determined that treatment of p53 wild type lung cancer cells with fenbendazole inhibits growth. The study evaluated growth of lung cancer cells or normal lung epithelium (NHBEC) after treatment with fenbendazole (labeled FEN in the figure) and other agents. Both H1299 and H322 are p53 deficient NSCLC cells and show modest growth inhibition by fenbendazole after 5-7 days. In contrast, the p53 wild type cells A549 and H460 show dramatic inhibition of cell growth by fenbendazole that is evident by day 1-3 and 50-80% growth inhibition by day 5-7 of treatment. The control normal cells, NHBEX do not show growth inhibition by fenbendazole. We generated the results of this study prior to March 9, 1999.

7. All work disclosed in the invention disclosure form was conducted in the United States of America.

8. Therefore, the invention as reflected in the claims of the above-referenced patent application was reduced to practice prior to March 9, 1999.

9. We hereby declare that all statements made by our own knowledge are true and all statements made on information and belief are believed to be true and further that statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment under § 100 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date

Tapas Mukhopadhyay

Date

Sunil Chada

Date

Abner Mhashilkar

Date

Jack A. Roth

Exhibit 1

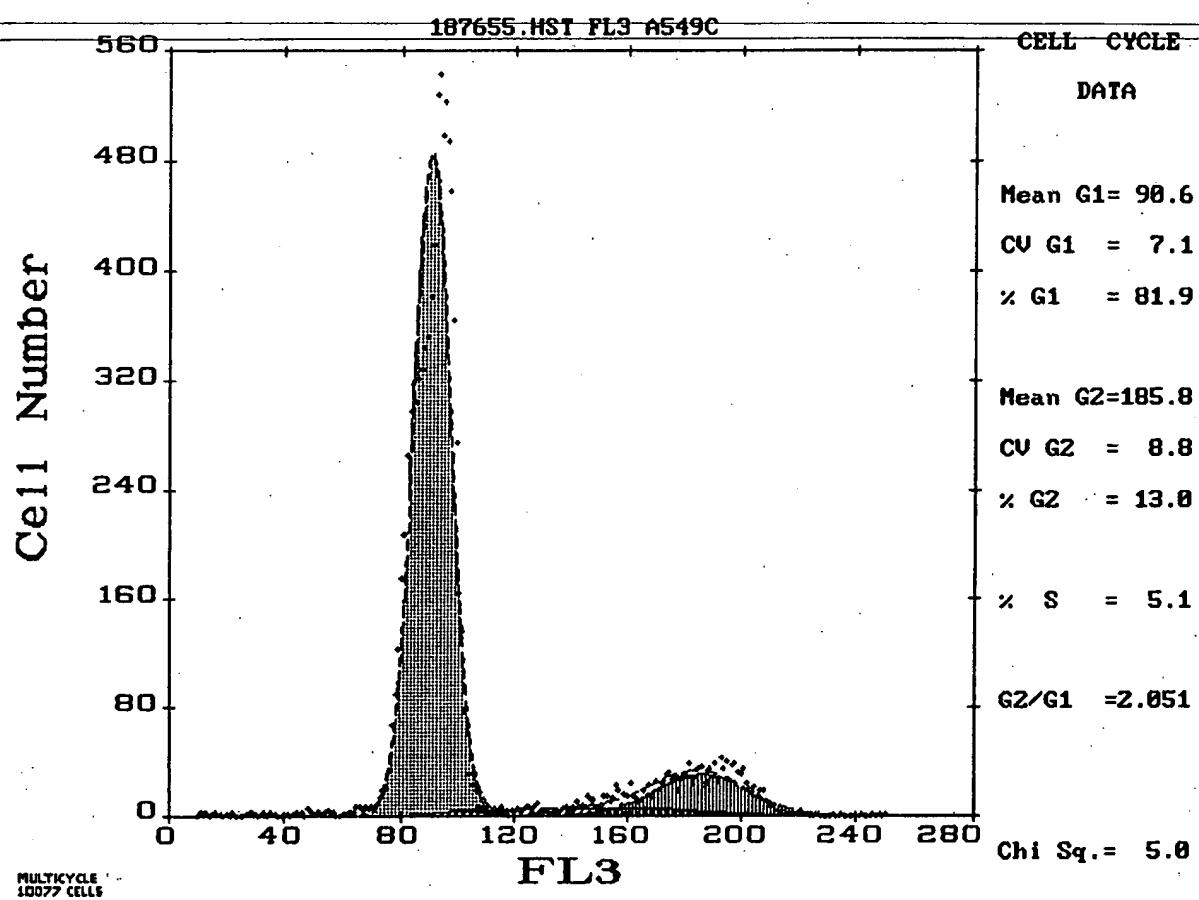


FIG. 1A

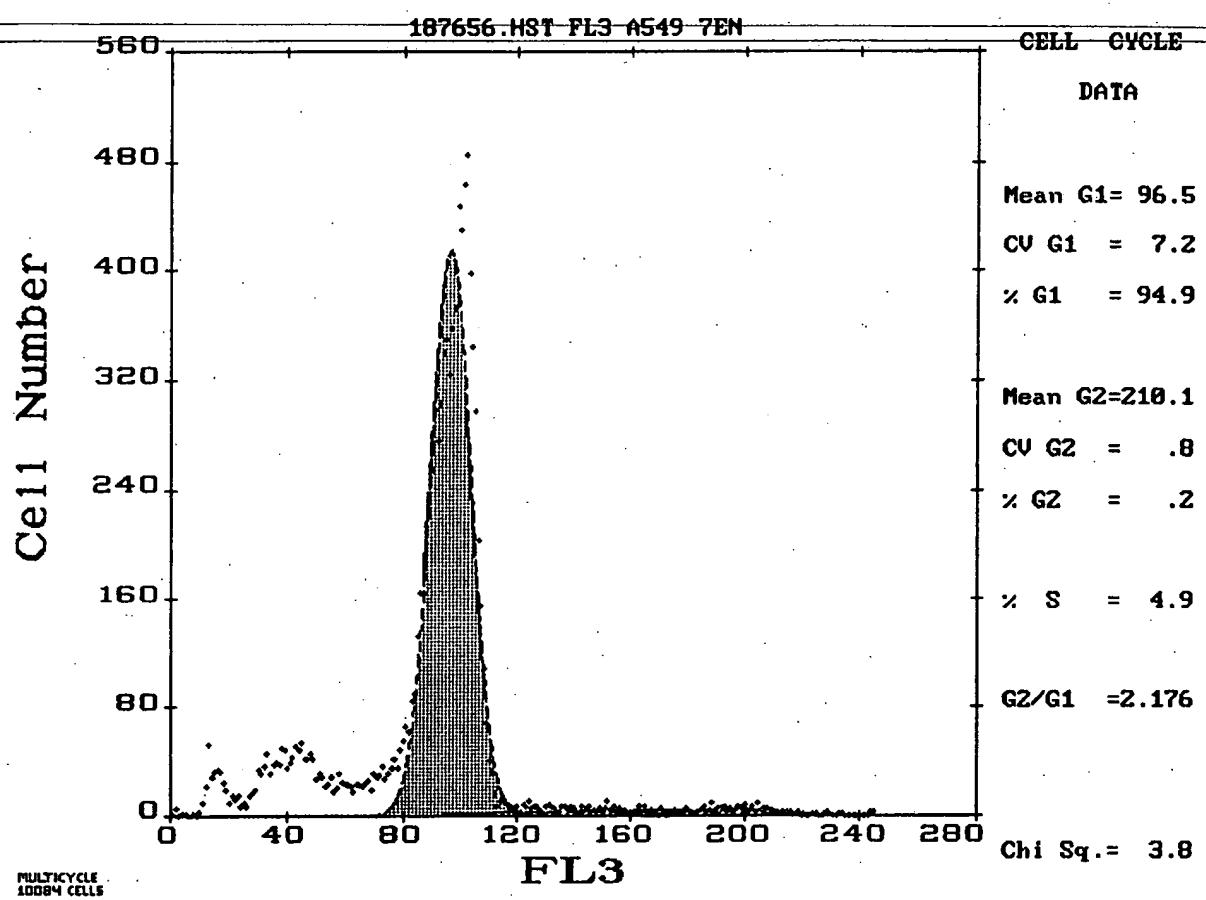
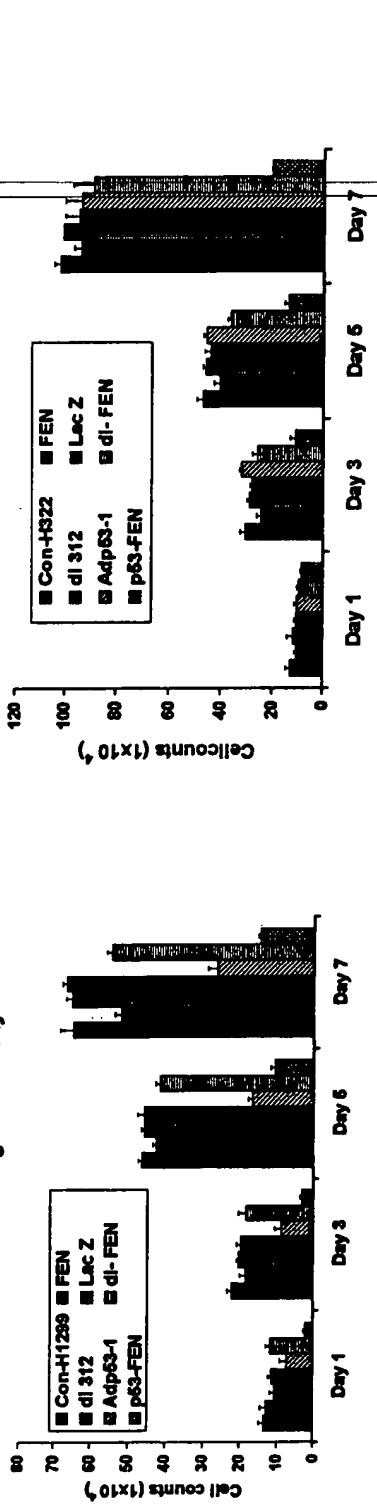


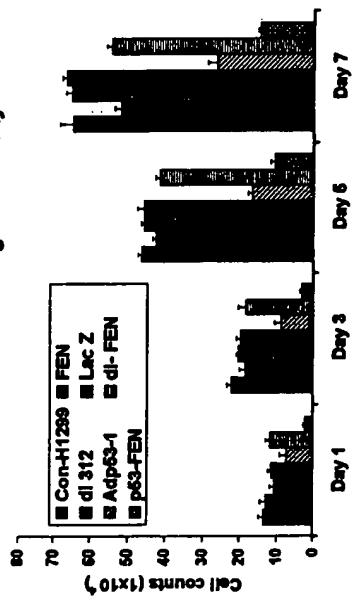
FIG. 1B

Exhibit 2

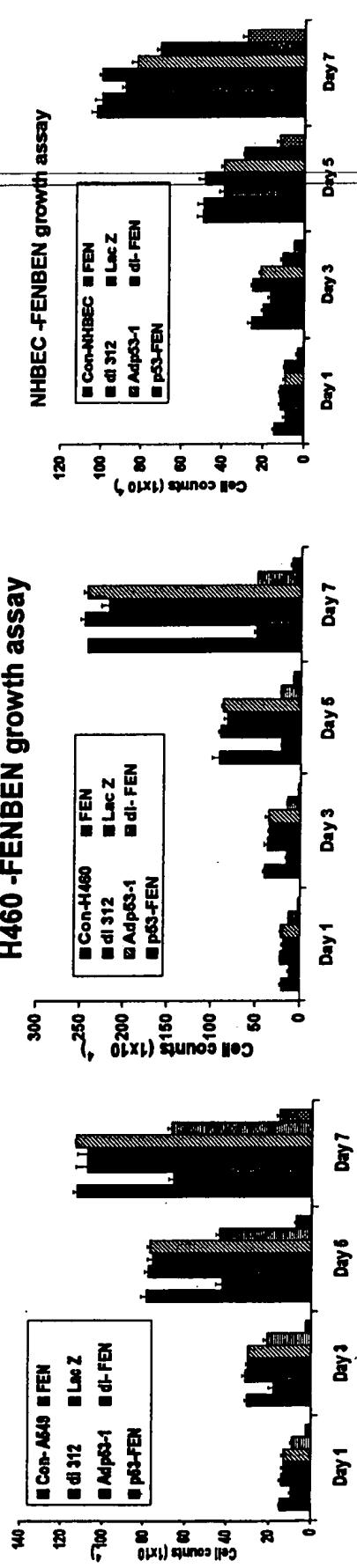
H322-FENBEN growth assay



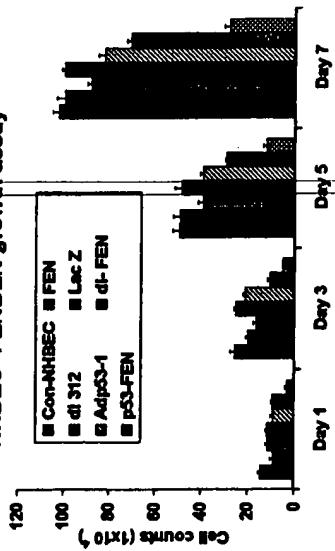
H1299-FENBEN growth assay



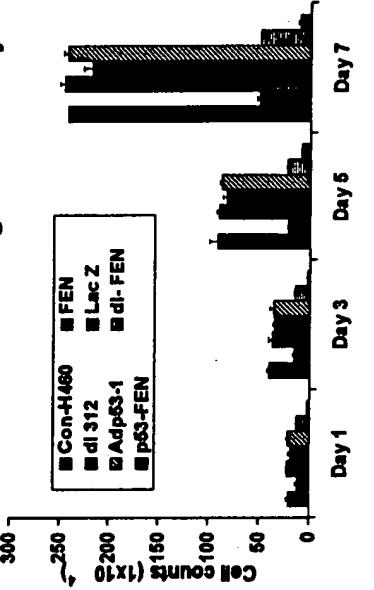
A549-FENBEN growth assay



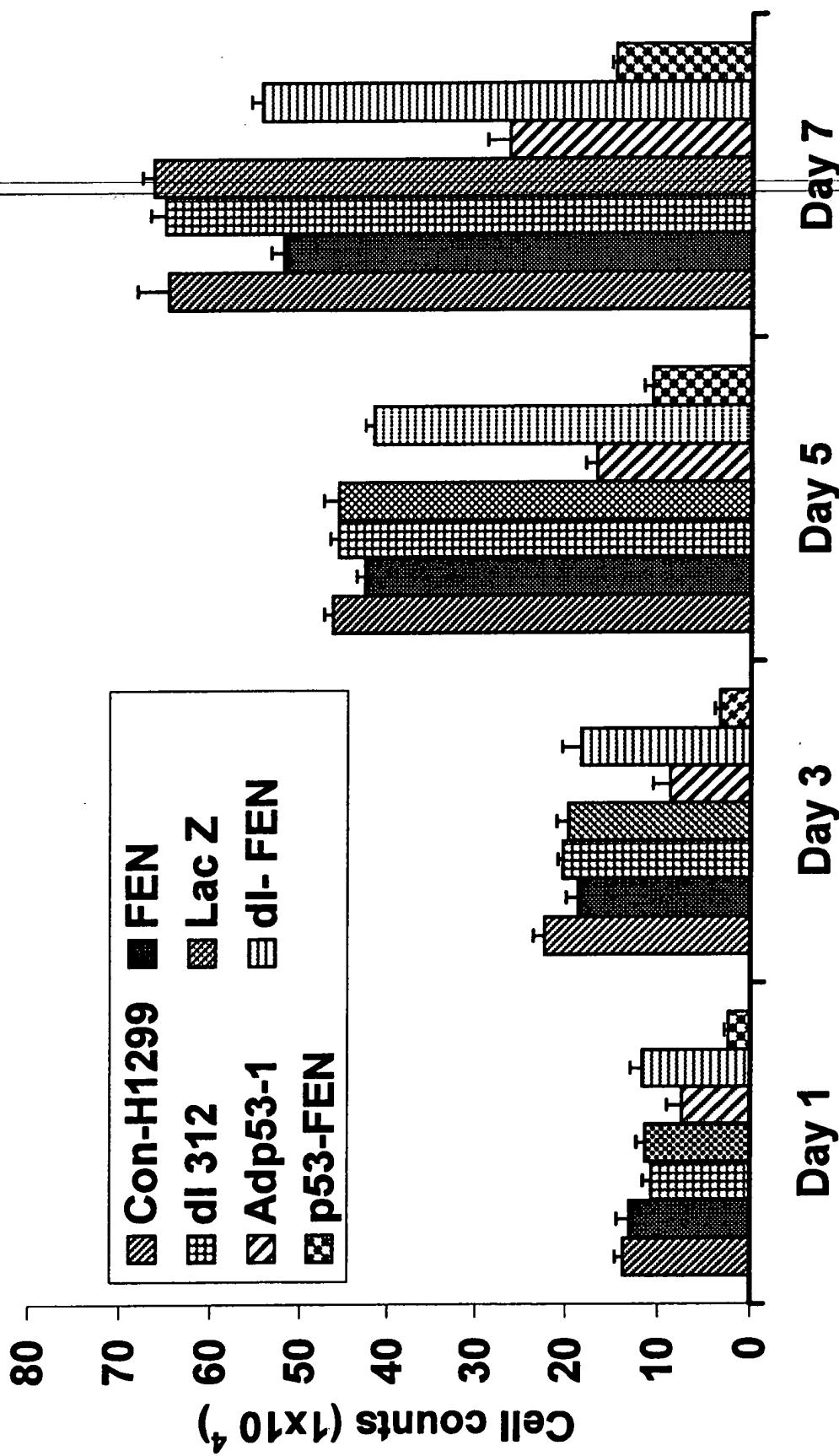
NHBECK-FENBEN growth assay



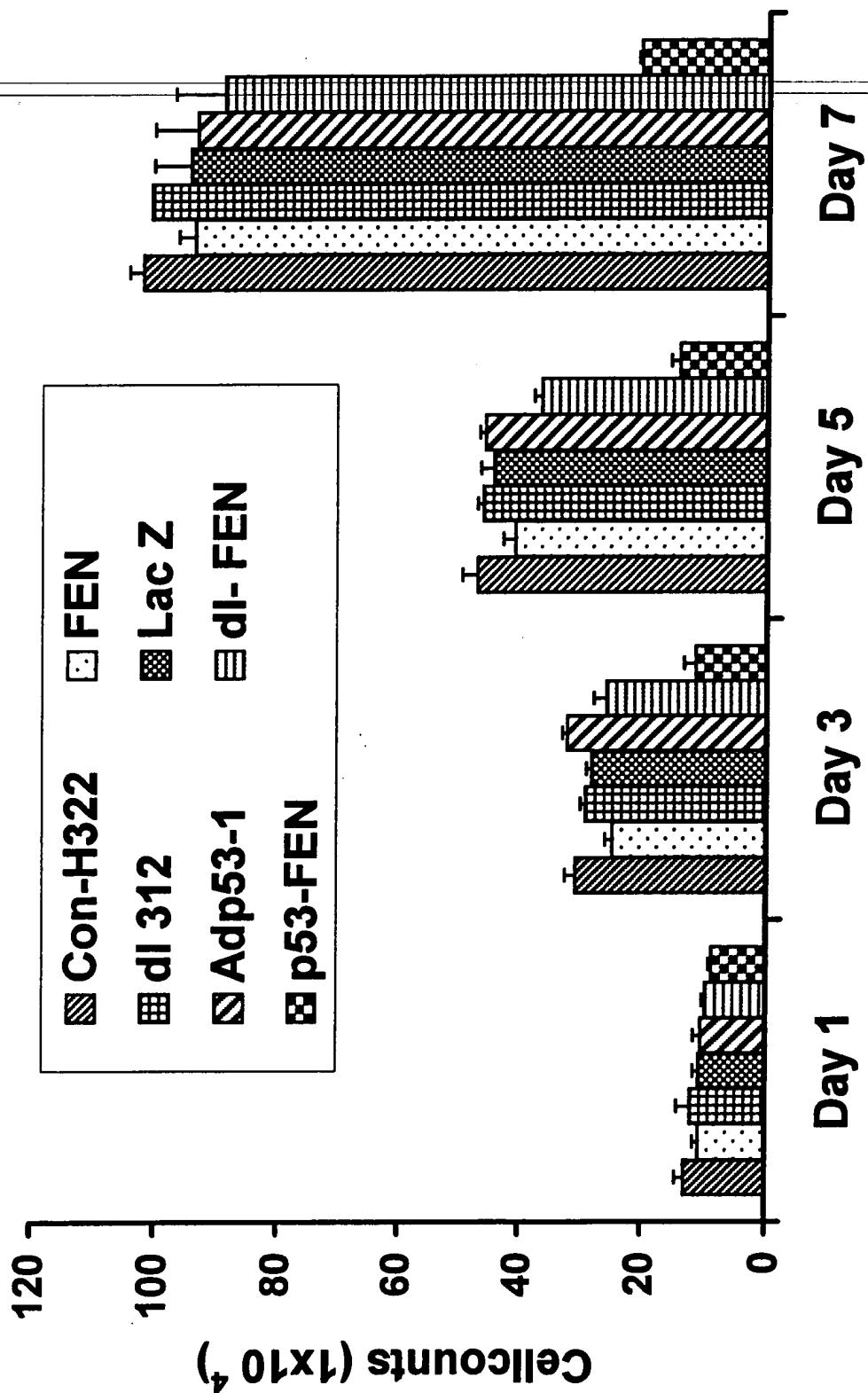
H460 -FENBEN growth assay



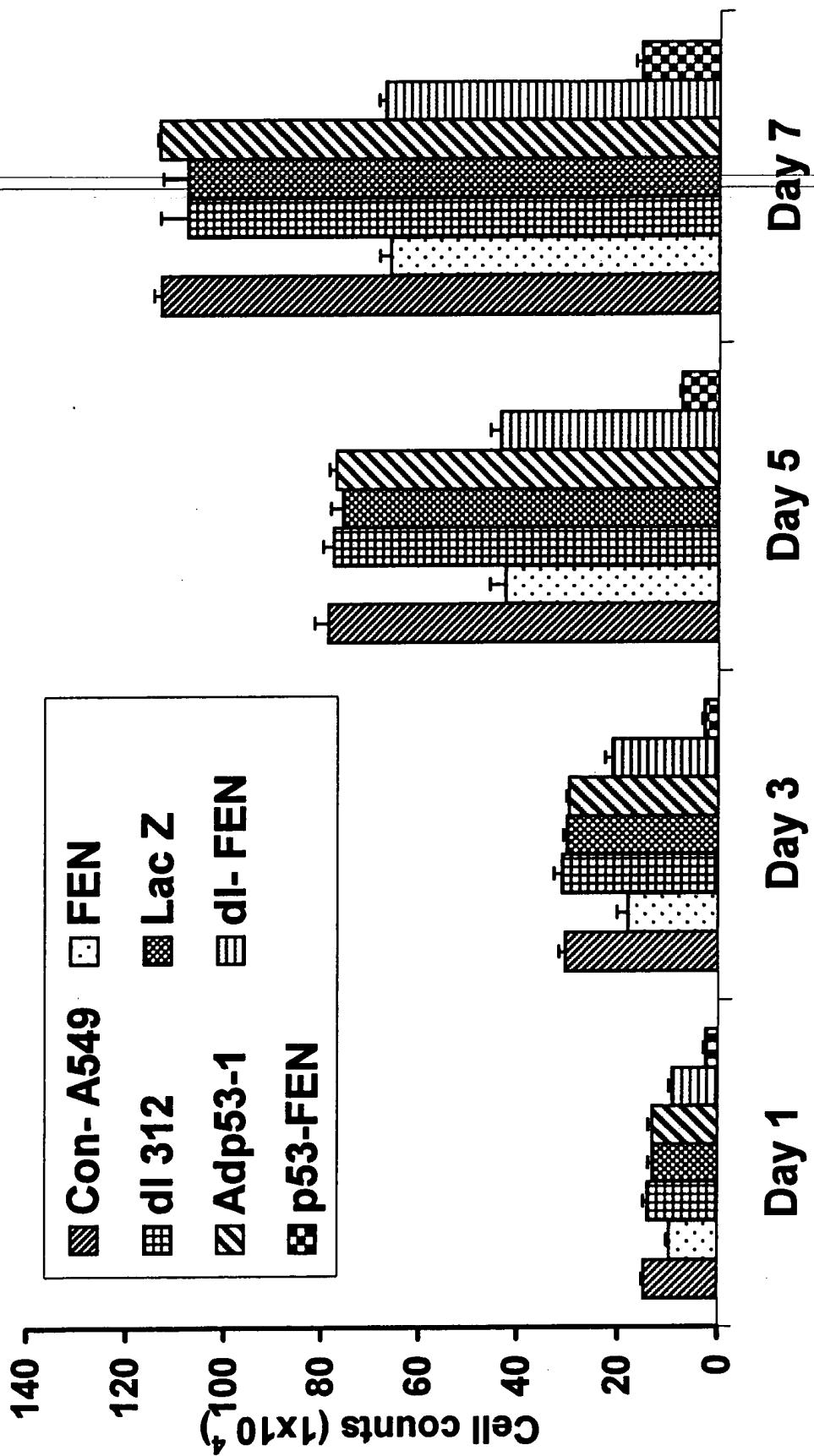
H1299-FENBEN growth assay



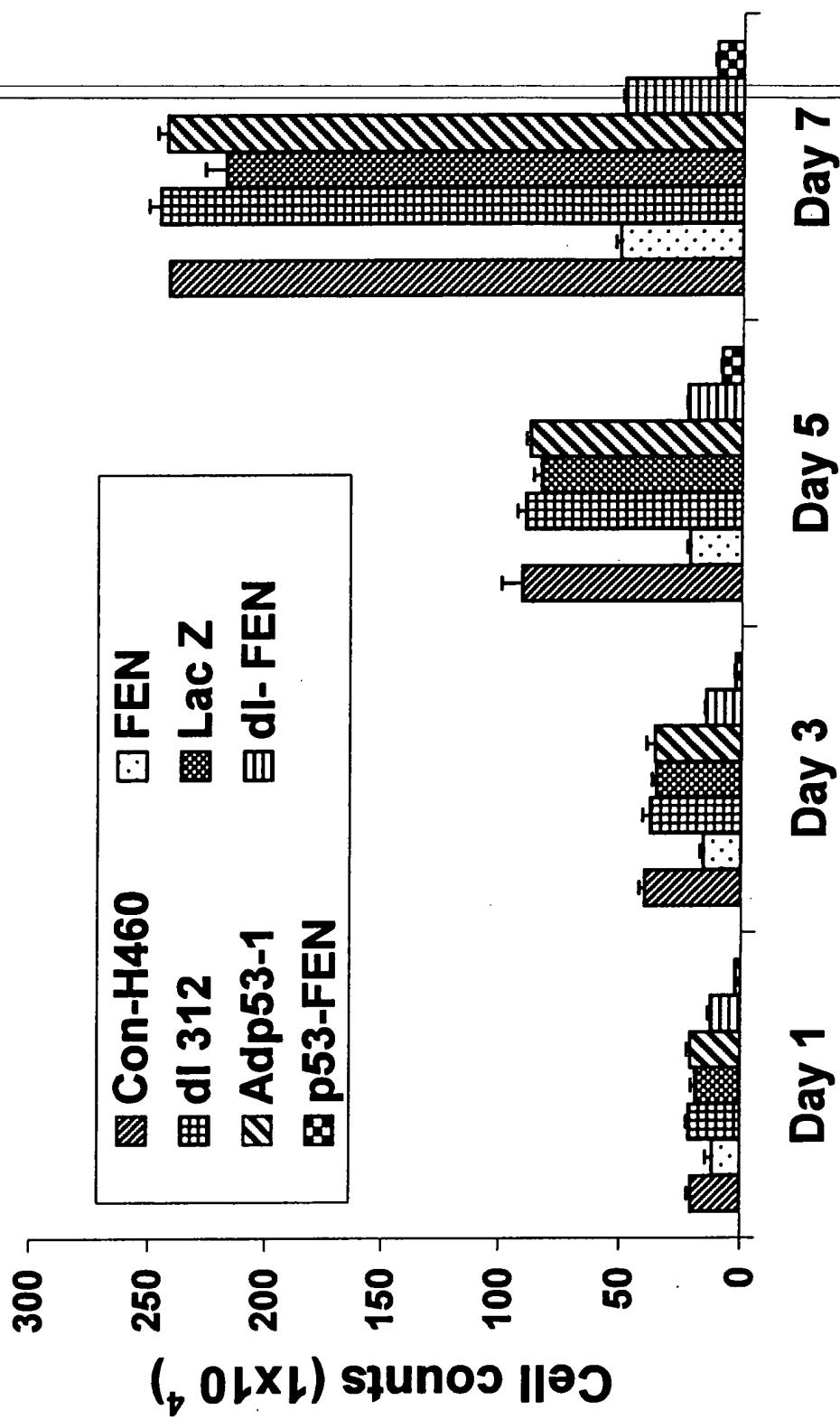
H322-FENBEN growth assay



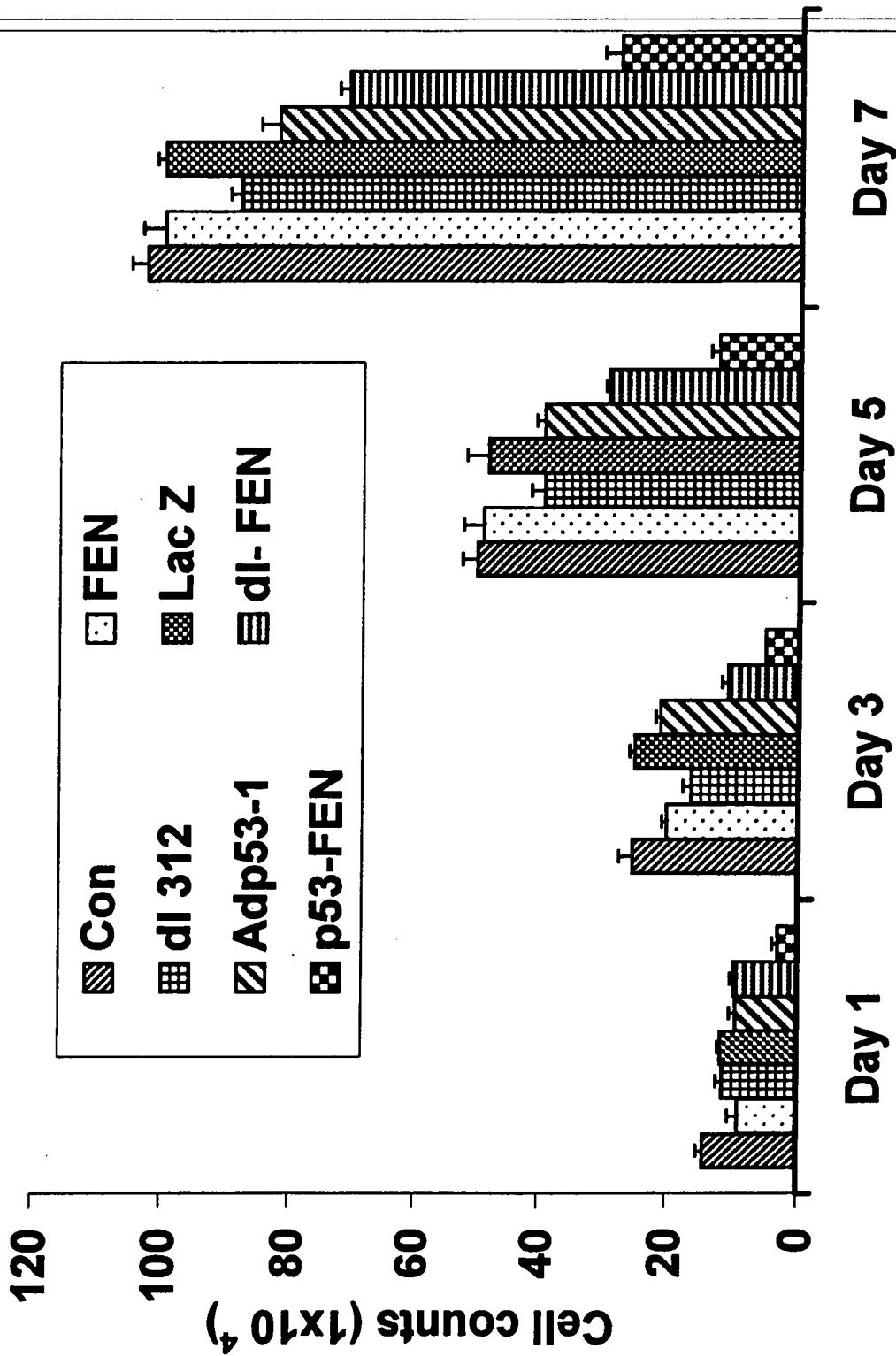
A549-FENBEN growth assay



H460 -FENBEN growth assay



HNBEC -FENBEN growth assay



NHBE

7-en 105mg
 edrods 12 mos = 1
 V3 mol = 1

	Day 1	Day 3	Day 5	Day 7	9
1	x2	x2	x3	x5	
2	30	15	50	25	70
3	26	13	46	23	67
4	30	15	56	28	62
5	x2	x2	x3	x5	
6	20	10	42	21	70
7	14	7	40	20	65
8	19	10	38	19	60
9	x2	x2	x3	x5	
10	19	10	32	16	56
11	24	12	30	18	52
12	23	12	35	18	50
13	x2	x2	x3	x5	
14	22	11	48	24	71
15	24	12	52	26	62
16	24	12	50	25	60
17	x2	x2	x3	x5	
18	20	10	40	20	51
19	15	8	42	21	55
20	19	10	44	22	52
21	x2	x2	x3	x5	
22	19	10	20	10	38
23	17	9	20	10	48
24	20	10	24	12	39
25	x1	x1	x2	x3	
26	17	4	18	5	22
27	10	3	19	5	27
28	9	2	19	5	24
29	V-F				
30	10	3	19	5	27
31	9	2	19	5	24

H460

	Day 1	Day 3	Day 5	Day 7	
Control	25	19	x3	x5	x10
x3	29	22	52	39	98
3	26	20	56	42	100
4			50	38	92
5	x3	x3	x3	x5	
6	18	14	21	16	40
7	17	13	19	14	38
8	10	8	22	17	42
9					53
10	x3	x3	x5	x10	
11	30	23	54	41	90
12	27	20	44	33	85
13	28	21	50	38	98
14					101
15	x3	x3	x5	x10	
16	21	16	49	37	78
17	27	20	47	35	85
18	26	20	44	33	90
19					122
20	x3	x3	x5	x10	
21	30	23	52	39	89
22	27	20	41	31	88
23	25	19	49	37	85
24					99
25	x3	x3	x3	x5	
26	15	11	20	15	22
27	18	14	20	15	22
28	16	12	18	14	30
29	x1	x1	x2	x2	
30	7	2	7	2	15
31	8	2	10	3	17
6	2	2	9	2	15
					8
					24
					12

171299

	Days		Days		Days		Days	
1	x2	x2	x2	x2	x3	x3	x3	x3
2	25	13	41	24	60	45	80	60
3	30	15	42	21	62	47	88	66
4	26	13	44	22	63	47	91	68
5	x2	x2	x2	x3	x3	x3	x3	x3
6	30	15	34	17	58	44	67	50
7	23	12	38	19	56	42	70	53
8	24	12	40	20	56	42	70	53
9	x2	x2	x2	x3	x3	x3	x3	x3
10	20	10	40	20	62	47	89	67
11	24	12	41	21	60	45	84	63
12	20	10	40	20	60	45	86	65
13								
14	x2	x2	x2	x3	x3	x3	x3	x3
15	23	12	41	21	59	44	86	65
16	20	10	39	20	60	45	90	68
17	24	12	36	18	64	48	88	66
18								
19	x2	x2	x2	x3	x3	x3	x3	x3
20	10	5	11	6	20	15	30	23
21	15	8	19	10	22	17	36	27
22	17	9	19	10	24	18	39	29
23								
24	x2	x2	x2	x3	x3	x3	x3	x3
25	20	10	32	16	55	41	72	54
26	23	12	42	21	57	43	74	56
27	25	13	36	18	55	41	70	53
28	x2	x1	x2	x2	x2	x2	x2	x2
29	9	2	10	3	20	10	30	15
30	8	2	12	3	20	10	27	14
31	13	3	16	4	24	12	29	15

A549

		Day 1	Day 3	Day 5	Day 7	
Central	x2	14	38	29	66	82
2	24	14	38	29	66	82
3	30	15	42	32	60	75
4	30	15	40	30	63	79
5	x2		x3	x5		x5
6	17	9	25	19	35	44
7	19	10	27	20	36	45
8	19	10	20	15	30	38
9						
d-E12	x2		x3	x5		x5
10	27	14	38	29	60	75
11	24	13	44	33	62	78
12	30	15	41	31	64	80
13						
14						
Angular	x2		x3	x5		x5
15	24	12	41	31	58	73
16	28	14	38	29	63	79
17	25	13	40	30	60	75
18						
19						
V3	x2		x3	x5		x5
20	28	14	40	30	62	78
21	26	13	40	30	62	78
22	24	12	39	29	60	75
23						
24						
d-F	x2		x3	x5		x5
25	18	8	28	21	34	43
26	17	9	30	23	37	46
27	20	10	25	19	33	41
28						
29						
V-F	x1		x1	x2		x3
30	10	3	10	3	14	7
31	8	2	12	3	16	8
8	2		9	2	13	1
						22
						17

H 322 J

		Day 1	Day 3	Day 5	Day 7	9
2	ontal	x2 29	x3 38	x5 66	x8 80	
3		15	29	50	100	
4		23	40	44	81	
5		12	30	59	101	
6		24	44	62	64	
7		12	33	47	105	
8	7em	x2 24	x3 33	x3 50	x5 74	96
9		10	25	38	76	
10		20	34	43	95	
11		10	26	72		
12		20	30	54	90	
13	11312	x2 20	x3 30	x3 54	x5 72	
14		11	40	41	80	100
15		24	41	47	80	100
16		12	35	60	82	102
17		13	36			
18	H-gal	x2 20	x2 36	x3 61	x5 79	99
19		10	27	46	78	
20		20	58	47	69	
21		10	28	44	86	
22		24	56	42		
23		12	39	62		
24		10	29	47		
25	x3	x2 20	x3 41	x3 62	x5 82	103
26		10	31	47		
27		18	60	45	71	89
28		9	42	44	69	
29		23	32	42	70	
30	d-7	12	40	45	70	88
31		12	30	35		
32		10	28	35		
33		10	46	35		
34		12	28	35		
35		10	46	35		
36		12	28	35		
37		10	46	35		
38		12	28	35		
39		10	46	35		
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242		12	28	35		
243		10	46	35		
244		12	28	35		
245		10	46	35		
246						

			Day 1	sd			
Con-NHB	15	13	15	14.33333	0.942809	25	23
FEN	10	7	10	9	1.414214	21	20
dl 312	10	12	12	11.33333	0.942809	16	15
Lac Z	11	12	12	11.66667	0.471405	24	26
Adp53-1	10	8	10	9.333333	0.942809	20	21
dl- FEN	10	9	10	9.666667	0.471405	10	10
p53-FEN	4	3	2	3	0.816497	5	5

			Day 1	sd			
Con-H460	19	22	20	20.33333	1.247219	39	42
FEN	14	13	8	11.66667	2.624669	16	14
dl 312	23	20	21	21.33333	1.247219	41	33
Lac Z	16	20	20	18.66667	1.885618	37	35
Adp53-1	23	20	19	20.66667	1.699673	39	31
dl- FEN	11	14	12	12.33333	1.247219	15	15
p53-FEN	2	2	2	2	0	2	3

			Day 1	sd			
Con- A549	14	15	15	14.66667	0.471405	29	32
FEN	9	10	10	9.666667	0.471405	19	20
dl 312	14	13	15	14	0.816497	29	33
Lac Z	12	14	13	13	0.816497	31	29
Adp53-1	14	13	12	13	0.816497	30	30
dl- FEN	8	9	10	9	0.816497	21	23
p53-FEN	3	2	2	2.333333	0.471405	3	3

			Day 1	sd			
Con-H322	15	12	12	13	1.414214	29	30
FEN	12	10	10	10.66667	0.942809	25	26
dl 312	11	12	13	12	0.816497	30	31
Lac Z	10	10	12	10.66667	0.942809	27	28
Adp53-1	10	9	12	10.33333	1.247219	31	32
dl- FEN	9	10	10	9.666667	0.471405	23	26
p53-FEN	8	9	9	8.666667	0.471405	10	10

			Day 1	sd			
Con-H129	13	15	13	13.66667	0.942809	24	21
FEN	15	12	12	13	1.414214	17	19
dl 312	10	12	10	10.66667	0.942809	20	21
Lac Z	12	10	12	11.33333	0.942809	21	20
Adp53-1	5	8	9	7.333333	1.699673	6	10
dl- FEN	10	12	13	11.66667	1.247219	16	21
p53-FEN	2	2	3	2.333333	0.471405	3	3

Day 3 sd			Day 5 sd		
28	25.33333	2.054805	53	50	47 50 2.44949
19	20	0.816497	53	49	45 49 3.265986
18	16.33333	1.247219	42	39	37 39.33333 2.054805
25	25	0.816497	53	47	45 48.33333 3.399346
22	21	0.816497	38	41	39 39.33333 1.247219
12	10.66667	0.942809	29	30	29 29.33333 0.471405
5	5	0	11	14	12 12.33333 1.247219

Day 3 sd			Day 5 sd		
38	39.66667	1.699673	88	94	90 90.66667 2.494438
17	15.66667	1.247219	21	23	20 21.33333 1.247219
38	37.33333	3.299832	90	85	93 89.33333 3.299832
33	35	1.632993	78	85	85 82.66667 3.299832
37	35.66667	3.399346	89	88	85 87.33333 1.699673
14	14.66667	0.471405	22	22	23 22.33333 0.471405
2	2.333333	0.471405	8	9	8 8.333333 0.471405

Day 3 sd			Day 5 sd		
30	30.33333	1.247219	82	75	79 78.66667 2.867442
15	18	2.160247	44	45	38 42.33333 3.091206
31	31	1.632993	75	78	80 77.66667 2.054805
30	30	0.816497	73	79	75 75.66667 2.494438
29	29.66667	0.471405	78	78	75 77 1.414214
19	21	1.632993	43	46	41 43.33333 2.054805
2	2.666667	0.471405	7	8	7 7.333333 0.471405

Day 3 sd			Day 5 sd		
33	30.66667	1.699673	50	44	47 47 2.44949
23	24.66667	1.247219	38	43	41 40.66667 2.054805
26	29	2.160247	46	47	45 46 0.816497
29	28	0.816497	47	44	42 44.33333 2.054805
33	32	0.816497	47	45	45 45.66667 0.942809
28	25.66667	2.054805	36	38	35 36.33333 1.247219
14	11.33333	1.885618	15	15	12 14 1.414214

Day 3 sd			Day 5 sd		
22	22.33333	1.247219	45	47	47 46.33333 0.942809
20	18.66667	1.247219	44	42	42 42.66667 0.942809
20	20.33333	0.471405	47	45	45 45.66667 0.942809
18	19.66667	1.247219	44	45	48 45.66667 1.699673
10	8.666667	1.885618	15	17	18 16.66667 1.247219
18	18.33333	2.054805	41	43	41 41.66667 0.942809
4	3.333333	0.471405	10	10	12 10.66667 0.942809

Day 7				sd
99	103	105	102.3333	2.494438
101	95	103	99.66667	3.399346
90	86	88	88	1.632993
100	101	98	99.66667	1.247219
83	78	85	82	2.94392
71	69	73	71	1.632993
24	29	30	27.66667	2.624669

Day 7				sd
245	250	230	241.6667	8.498366
50	48	53	50.33333	2.054805
240	245	252	245.6667	4.921608
205	225	223	217.6667	8.993825
238	242	248	242.6667	4.109609
48	50	48	48.66667	0.942809
10	10	12	10.66667	0.942809

Day 7				sd
111	113	115	113	1.632993
64	65	69	68	2.160247
100	110	113	107.6667	5.557777
113	109	101	107.6667	4.988877
113	114	113	113.3333	0.471405
68	65	68	67	1.414214
14	15	17	15.33333	1.247219

Day 7				sd
100	101	105	102	2.160247
96	95	90	93.66667	2.624669
100	100	102	100.6667	0.942809
99	98	86	94.33333	5.906682
103	89	88	93.33333	6.847546
80	99	88	89	7.788881
20	20	21	20.33333	0.471405

Day 7				sd
60	66	68	64.66667	3.399346
50	53	53	52	1.414214
67	63	65	65	1.632993
65	68	66	66.33333	1.247219
23	27	29	26.33333	2.494438
54	56	53	54.33333	1.247219
15	14	15	14.66667	0.471405

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